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We have continued our efforts to develop an effective HIV-1 vaccine based on the V1/V2 domain of gp120. Studies with human sera and with antibodies isolated from immunized animals have shown that the V1/V2 domain contains highly conserved epitopes that can act as potent neutralizing targets for a broad range of primary HIV-1 isolates. We have prepared a prototype immunogen, based on the Case-A2 isolate, that contains the clade B V2 consensus sequence, and have shown that this antigen induces highly crossreactive anti-V1/V2 antibodies in both rats and macaques, and that specific fractions of the rat antibodies possess potent cross-neutralizing activity. We are continuing to analyze this immunogen in both the rat and macaque models, and we expect to further analyze the humoral responses to immunization with this protein, including the isolation of monoclonal antibodies against various epitopes in the V1/V2 domain. Our goal during the coming year is to more fully characterize the epitopes mediating the potent neutralization, and to modify the immunogen so that those epitopes are presented more specifically and efficiently. The macaque immunizations will provide sufficient amounts of antibodies for full characterization, and will also allow challenge studies to be performed with appropriate SHIV strains.

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Abraham Rudin 8/7/97

PI - Signature Date

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DEVELOPMENT OF A V1/V2-BASED VACCINE AGAINST HIV-1

Introduction

There is presently a dearth of candidate HIV vaccines that are considered suitable for wide-scale testing in humans, and consequently there is much pessimism about the possibility for a successful vaccine for HIV-1 in the near future. Whereas live, attenuated viruses may provide protection against more pathogenic strains, safety considerations are likely to preclude the wide-spread use of such vaccines. A difficulty with purified envelope subunit vaccines is that while the best of these have been able to induce neutralizing responses against the vaccine strain and related laboratory-adapted, T cell-tropic HIV-1 strains, these vaccines have not induced neutralizing responses to primary viruses and clinical HIV-1 isolates (6, 11, 12). This finding may be related to the general resistance of primary viruses to neutralization by sCD4 (1, 4), monoclonal antibodies (2, 14), and immune sera from many HIV-infected patients (3). The reason for the difference in sensitivities of primary viruses and lab isolates is not clear. It has been suggested that epigenetic factors related to the cells the virus was prepared in (17) and to the incorporation of host cell adhesion proteins into virion membranes (5, 7) may be involved. Our own data showing dramatically different sensitivities to neutralization of a pair of related molecularly cloned T cell-tropic and macrophage-tropic viruses prepared in the same cells and assayed in a common batch of activated PBMCs indicates that this phenomenom is genetically determined, related to tropism, and is directly determined by the structure and sequence of the virus Env protein.

Whereas it is known that some people possess potent neutralizing antibodies against primary strains of HIV, such activities are rare, and the nature of the epitopes that mediate this activity are generally unknown. We have identified multiple human sera that possess highly cross-reactive anti-V1/V2 antibodies, and have shown that specific fractions of these antibodies possess potent neutralizing activities against both homologous and heterologous macrophage-tropic viruses (8, 16, 18). Based on these results, we have been developing a new approach towards the development of an effective HIV vaccine that involves the preparing of a recombinant protein that expresses the native V1/V2 domain of HIV-1 gp120 in immunogenic form. We have obtained encouraging results in rodent immunizations with this immunogen which indicate that we can induce antibodies directed against highly conserved epitopes in V1/V2 that possess potent neutralizing activities for a broad range of primary HIV-1 isolates. We have recently initiated primate immunization studies with this immunogen. During the coming year we expect to continue these immunization studies and to further improve the design of our immunogen.

PROGRESS REPORT

1. Characterization of a V1/V2 fusion protein suitable as a vaccine candidate

Our previous studies with both monoclonal and polyclonal antibodies have demonstrated the presence of epitopes in the V1/V2 domain of HIV-1 gp120 that can mediate potent neutralization of primary isolates of HIV-1. We were therefore interested in determining whether a vaccine based on V1/V2 fusion proteins similar to those described in the above studies would be able to inducing crossreactive antibodies capable of neutralizing diverse primary viruses. One difficulty encountered towards this approach was that the V1/V2 domain is highly conformational, and our initial constructs contained a large fraction of misfolded molecules that failed to express native conformational epitopes (21). In addition, the HXB2 sequence initially used contained a number of rare substitutions at several polymorphic sites in V2, which resulted in the presence of a number of type-specific epitopes (13, 15, 21). We therefore searched for another V1/V2 sequence that contained a more representative V2 sequence and which folded more efficiently.

Wang et al. have characterized the V1/V2 domains of 57 HIV-1 sequences obtained from a group of 47 HIV-infected individuals (19). Among these sequences was the Case-A2 isolate, obtained from an infected infant, that possessed essentially the North American clade B consensus V2 sequence (Fig. 1). We have expressed this V1/V2 domain as a gp70 fusion protein in the Celltech pEE14 vector and have examined its utility as an immunogen.

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- - - - S - - - - G - - - - - - - - F - - - - - I - - - - HXB2
- - - - - - - - - - - - - - - - - - - - - - - - I - - - - Case-A2

S F N I T T S I R D K V Q K E Y A L F Y K L D V V P I D  Most common clade B
55 55 46 42 46 54 43 45 47 31 52 32 45 50 45 47 55 40 51 47 42 52 55 33 47 54 52 44  /55

* * Y V S   N R G N R M K R Q N * F L N R Y * I I S V E  2nd most common
    4 11 8 1 7 5 5 18 2 13 6 2 3 4   10 4 4 7 1   21 3 1 1 5  /55
|                                                          |
158                                                        185
```

Fig. 1. Comparision of the HXB2 and Case-A2 V2 region sequences with the most common sequences in this region derived from a database of 55 North American isolates shows the similarity of the Case-A2 sequence with the clade B consensus sequence in this region. The first and second most common residues at each position are given, and frequency of a particular residue at a given position is indicated beneath the consensus sequences.

2. Structure of Case-A2 V1/V2 fusion protein

The Case-A2 V1/V2 domain was expressed as a fusion protein, joined to the C-terminus of a fragment (residues 1-263) of the MuLV gp70 protein (9). A His6 affinity signal was inserted at position 9 of the gp70-derived sequence, thereby allowing a simple single step purification on Ni-NTA columns (Fig. 2). The fused gene was expressed in the pEE14 vector; this vector also expresses the glutamine synthetase gene, which allows selection of transfected cells by growth in glutamine deficient medium in the presence of the glutamine antagonist, MSX. This vector was transfected into CHO cells, and clones producing the protein were isolated in the selecting medium. Positive clones were identified by ELISA, and the proteins secreted by these cells were characterized by radioimmunoprecipitation assays and SDS-PAGE.

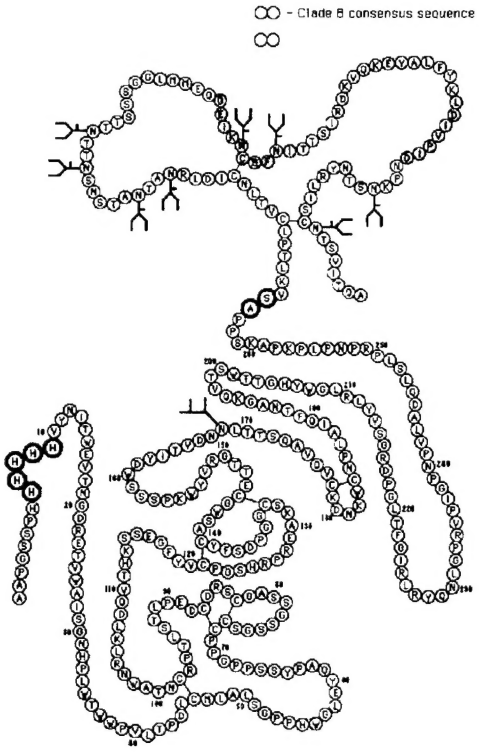


Fig. 2. Structure of Case-A2 V1/V2 fusion protein (p565) used as immunogen. Amino acids 111-206 of the Case-A2 V1/V2 domain were fused to the C-terminus of a 263 amino acid N-terminal fragment of the MuLV SU protein, gp70. A His6 signal was iserted near the N-terminus of the gp70 region to provide an affinity tag that allows facile purification of the recombinant protein from supernatants of stably transfected CHO cell lines.

Initial studies have indicated that the Case-A2 V1/V2 fusion protein was folded more heterogenously than our initial V1/V2 fusion protein, derived from the HXB2 sequence. However, more careful analyses showed that this protein was also present in at least two immunologically distinguishable forms which could be distinguished by their reactivity with different monoclonal antibodies. Sequential radioimmunoprecipitation experiments with several rat monoclonal anti-V1/V2 antibodies directed against linear epitopes showed that these antibodies recognized a distinct fraction of the protein. Two mabs, C9B6 and K19B3, directed against linear epitopes recognized approximately half of the protein, while two mouse mabs, 238 and 258, directed against conformational epitopes, reacted predominantly with the other half. This suggests that only half of the molecules were correctly folded and presented native conformational epitopes, while the other half was misfolded and presented only linear or misfolded epitopes. The fact that the monoclonal antibodies isolated to date after immunization with the V1/V2 fusion protein were directed against the linear epitopes suggested that the nonnative fraction was more immunoreactive than the correctly folded fraction of the protein.

3. Immunizations of rats with V1/V2 fusion proteins

Initially our emphasis was to determine an efficient adjuvant and dosing schedule that elicited an efficient humoral immune response to V1/V2 fusion proteins, in order to examine the specificities and functional activities of the resulting antibodies. A number of 2-4 month old female Fischer F344 rats were immunized with purified fusion glycoproteins expressing the V1/V2 sequences of either HXB2 or Case A2 envelope gp120. The immunogens were formulated with either QS21 (from CBCX, Inc.) or RAS MPL+TDM (from Ribi Immunochemicals, Inc.) adjuvant in a format recommended by

the manufacturers. Three rats in each group were immunized with immunogens at 5 μg/rat and were boosted in the same formulation 6 weeks later at a dosage of 1μg/rat. Rats were boosted again at a 5-6 weeks interval with the same dosage and formulation and bled 1 week post each boost, and analyzed by ELISA against various antigens.

These results showed that the Case A2 V1/V2-B protein was a more effective immunogen than the HXB2 protein. Sera from both RAS and QS21 adjuvant groups were analysed for their crossreactivity against purified envelope proteins derived from LAV, MN and CM strains, using equal amounts of each envelope protein (not shown). In general, the group immunized with Case A2 immunogen produced higher titers and better crossreactivity than those immunized with the HXB2 protein. All three rats immunized with the Case-A2 protein produced antibodies that crossreacted with LAV gp120, and several of the immunized animal sera demonstrated appreciable titers to MN gp120. These results indicated that despite the purported hypervariability of the V1/V2 region, the Case-A2 immunogen was able to produce respectable titers against unrelated gp120s that contained a heterologous V1/V2 sequence. Furthermore, since the gp120s used was presumably correctly folded material produced in CHO cells, the crossreactive anti-V1/V2 antibodies present in the immune rat sera were apparently recognizing conserved, native epitopes in this domain.

4. Epitope specificity of anti-V1/V2 antibodies induced by Case-A2 V1/V2 fusion proteins

Western blots assays indicated that the sera of some rats immunized with V1/V2 fusion proteins contained antibodies that reacted with denatured V1/V2 fusion proteins. In order to map the epitopes recognized by these antibodies, a set of 15-mers that overlapped by approximately 5 residues and that represented the entire Case-A2 V1/V2 sequence were synthesized (Fig. 3). ELISA assays with these peptides showed that five of seven rats immunized twice with the immunogen the linear epitopes recognized were localized to a single peptide, peptide 7, that corresponded to the most highly conserved region of the V2 domain. Two other rats immunized seven times with the Case-A2 immunogen also recognized only this peptide. In contrast, a screen of 100 sera of HIV-infected humans identified only one that reacted with this peptide. This suggested that this sequence, while highly conserved, was not very immunogenic when expressed during HIV infection, but was immunogenic when presented as the Case-A2 fusion protein.

Case-A2 V1/V2 sequence:

VKLTPLCVTLNCIDLRNATNATSNSNTTNTTSSSGGLMMEQGEIKNCSFNITTSIRDKVQKEYALFYKLDIVPIDNPKNSTNYRLI

		OD405 (range)
p1	asVKLTPLCVTLNSI	0.05 (0-0.38)
p2	VTLNCIDLRNATNAT	0.01 (0-0.09)
p3	ATNATSNSNTTNTTS	0.01 (0-0.06)
p4	TNTTSSSGGLMMEQG	0.02 (0-0.16)
p5	MMEQGEIKNCSFNIT	0.00
p6	SFNITTSIRDKVQKE	0.00
T15K	TTSIRDKVQKEYALFYK	1.39 (0.74-1.99)
p7	SIRDKVQKEYALFYK	1.04 (0.39-1.46)
p8	EYALFYKLDIVPIDN	0.00

Fig. 3. The linear epitope recognized by rats immunized with Case-A2 V1/V2 fusion protein maps to the conserved V2 domain that contains known neutralizing epitopes

Peptide analogues of the reactive sequence were prepared in large scale, to allow further characterization of the antibodies induced against this sequence. To increase peptide solubility and to

facilitate the immobilization of the peptide two additional lysine residues were introduced at the N-terminus, followed by two naturally occurring threonines. The resulting peptide was called T15K, and has the sequence kkTTSIRDKVQKEYALFYK. The specificities of the antibodies recognizing this peptide epitopes were further defined by analyzing a series of N-terminal and C-terminal truncations of the T15K peptide (Fig. 4). Peptides that expressed the epitope, as defined by retention of reactivity by the majority of rat sera, are indicated in red. Deletion of the C-terminal lysine did not affect the epitope, while deletion of an additional two hydrophobic residues, phenylalanine and tyrosine, resulted in a reduction of binding, and deletion of an additional leucine resulted in loss of recognition by all but one serum. N-terminal deletions of the two threonines retained reactivity, while further deletion of serine and isoleucine led to the complete loss of reactivity of both sera. Thus, the minimal epitopes recognized were located in the sequence (S)IRDKVQKEYAL(FY), with a decreased or unknown effect of the terminal residues in parentheses. Interestingly, this sequence partially overlapped with the homologous peptide determinant (STSIRGKV) of the strongly neutralizing C108G MAb (20), suggesting that this region may also be a neutralization epitope.

Peptide	**Sequence**	**OD405**
	C-terminal deletions	Avg of 6 rats (range)
T15K	kkTTSIRDKVQKEYALFYK	1.34 (0.91-1.92)
pep1	kkTTSIRDKVQKEYALFY	0.86 (0.49-1.53)
pep2	kkTTSIRDKVQKEYAL	0.59 (0.02-1.40)
pep2a	kkTTSIRDKVQKEYA	0.35 (0.02-1.38)
pep3	kkTTSIRDKVQKEY	0.23 (0.02-0.81)
	N-terminal deletions	
pep4	kk--SIRDKVQKEYALFYK	0.99 (0.02-1.40)
pep5	kk----RDKVQKEYALFYK	0.24 (0.02-0.94)
pep6	kk-----DKVQKEYALFYK	0.09 (0.02-0.38)
C108G	STSIRGKV	

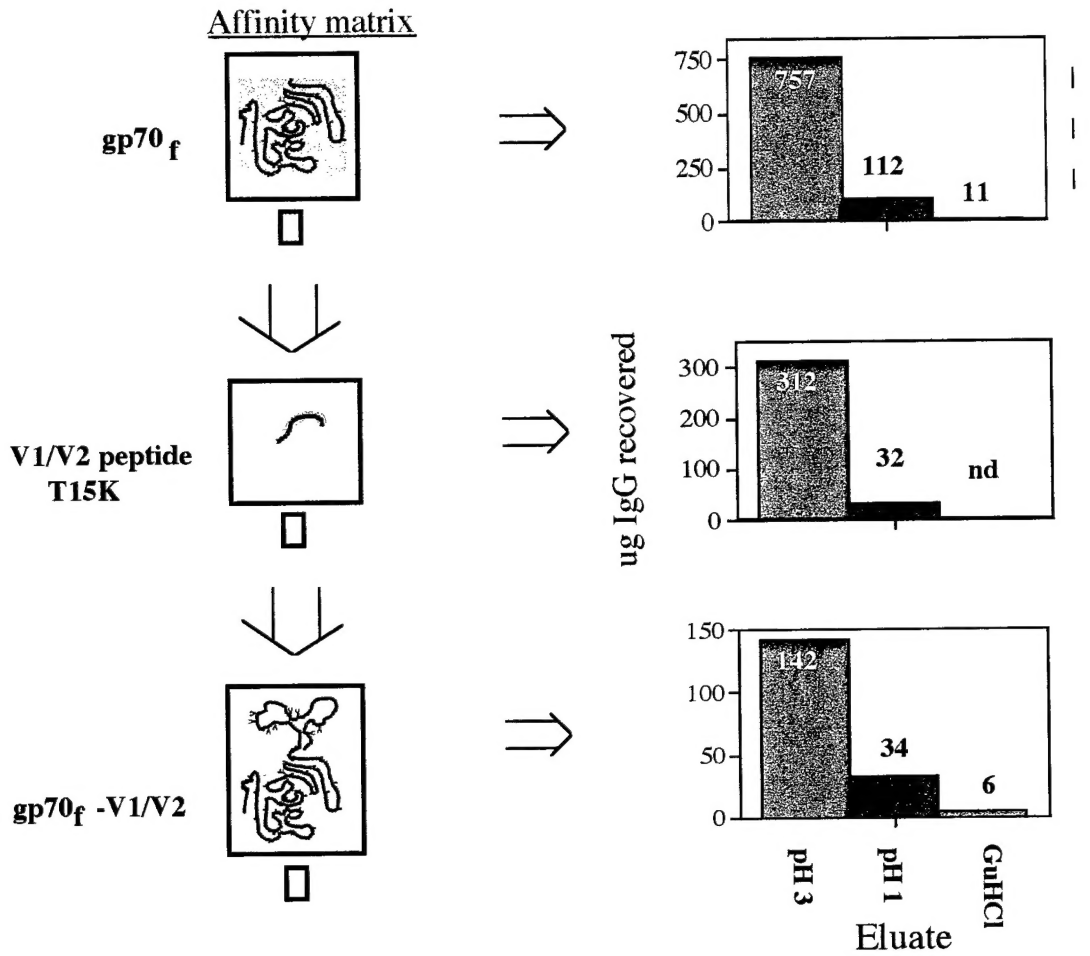
Fig. 4. Epitope mapping of rat antibodies directed against the V2 peptide T15K. Elisas were performed with serum dilutions of 1:100.

5. Neutralization activities of anti-V1/V2 antibodies produced by immunized rats

Preliminary experiments indicated that the sera of all of the immunized rats possessed neutralization activities against a number of HIV-1 isolates. In order to evaluate the role and potency of the anti-V1/V2 antibodies present in these sera, and to eliminate background effects to nonspecific components present in the rat sera, the immunoglobulins were sequentially fractionated by affinity chromatography on several antigen columns (Fig. 5). The serum was first absorbed on a column containing immobilized gp70 fragment, to remove the irrelevant anti-gp70 antibodies. The residual antibodies were then absorbed on a T15K peptide column, and specifically bound antibodies were eluted sequentially with pH 3 buffer, pH 1 buffer, and buffer containing the denaturant 5M GuHCl. The unabsorbed flow-through of this column was then fractionated on a Case-A2 V1/V2 fusion protein column, and specifically bound antibodies eluted by the same set of eluants. In this way, we were able to separate the peptide-specific antibodies from additional antibodies that may be reactive with conformational epitopes.

Specifically bound antibodies were eluted from each column. For the serum of one rat immunized seven times at roughly monthly intervals a total of 1.4 mg of antibody was recovered. The

Fig. 5 Analysis of specific anti-V1/V2 antibody fractions of immune rat serum sequentially eluted from V1/V2 affinity column



majority of this antibody (63%) was directed against the gp70-derived sequences, about 24% was directed against the T15K V2 peptide and 13% against conformational V1/V2 epitopes. Interestingly, a similar fractionation of the serum of another rat immunized only twice gave a higher percentage and yield of antibodies against the conserved conformational epitopes, suggesting that the efficiency of these immunizations would be improved by more appropriate timing of boosts. The neutralizing activities of these samples were compared to those of the starting serum and protein G-purified IgG sample.

The V1/V2-specific antibodies possessed potent neutralization activities for a number of primary macrophage-tropic viruses (Fig. 6). The GuHCl eluate of the V1/V2 fusion protein column had the most potent activity, but the pH3 eluate of the T15K also had significant neutralizing activity. These antibody fractions neutralized a number of all primary viruses assayed, including primary isolates of clades B, C, D and E. In each case the anti-V1/V2 antibodies were approximately 10-fold more potent that the anti-T15K peptide antibodies; ND50 values were in the range of 0.03-0.24 μg/ml for the anti-V1/V2 antibodies (with the exception of Th024E, for which an ND50 was not obtained) and in the range of <0.8-12 μg/ml for the anti-T15K peptide antibody fraction. As an example, these antibody fractions neutralized a primary clade D isolate from Uganda, Ug005-D with ND90 values of 1.4 mg/ml for the T15K antibodies and 0.20 mg/ml for the GuHCl eluate of the V1/V2 column. In addition to the viruses shown in this figure, the GuHCl eluate of the V1/V2 fusion protein column also neutralized a clade B clinical isolate, US716B (10), with an ND50 of 0.03 mg/ml, while the T15K pH3 eluate also was able to neutralize this virus, but with about an order of magnitude lower potency (ND50 of <.80 mg/ml).

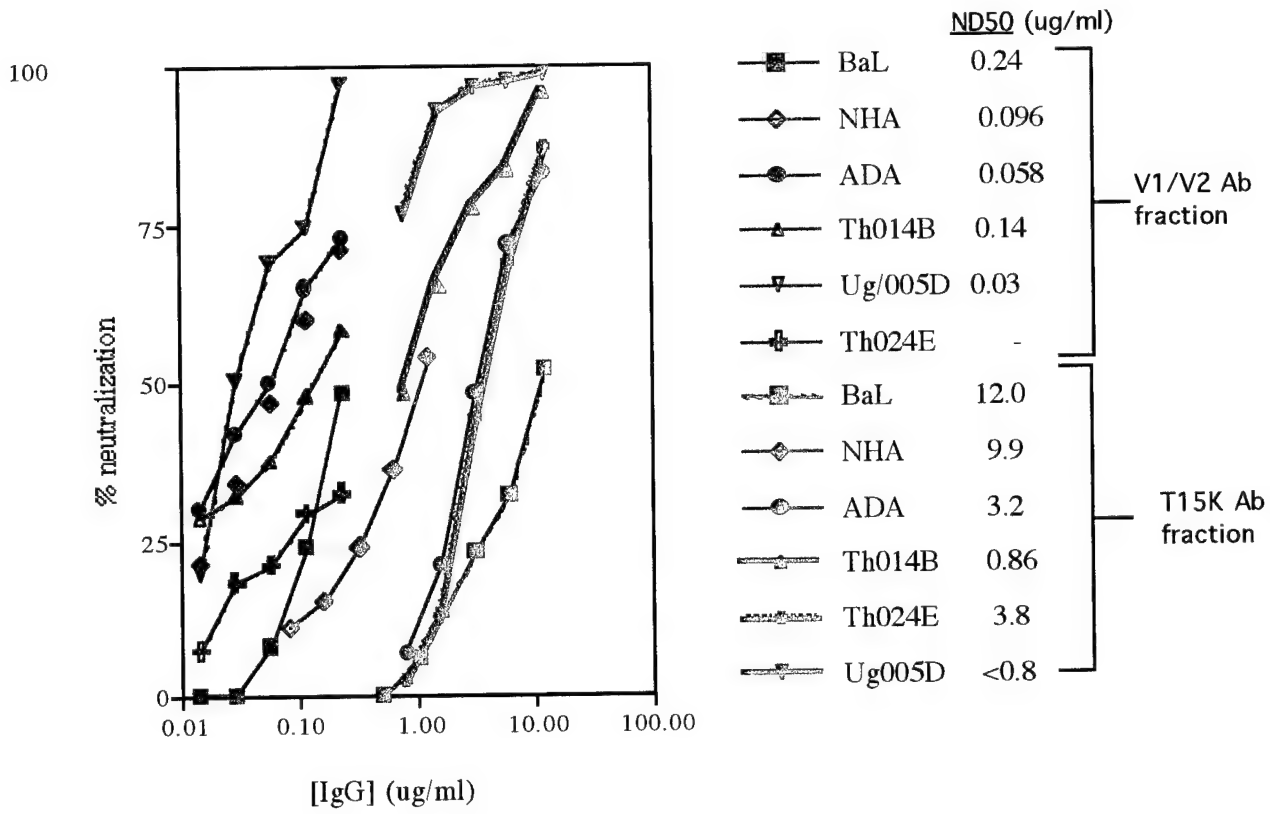


Fig. 6. Neutralization curves for rat antibodies recovered from V1/V2 affinity columns tested against primary HIV-1 isolates of multiple clades. Curves in blue are for antibodies recovered in the 5M GuHCl eluate of the Case-A2 V1/V2 column, while curves in red are for the pH 3 eluate of the T15K peptide column

6. Immunization of rhesus macaques with a V1/V2 fusion protein

We have started to immunize rhesus macaques with our purified Case-A2 V1/V2 fusion protein. These experiments are being performed in collaboration with Dr. Mark Lewis of the Henry M. Jackson Foundation, using animals that are housed at the Frederick Cancer Center. Dr. Lewis has an NIAID-funded contract to perform such vaccine evaluations, and the NIH is covering all of the animal costs, as well as Dr. Lewis' expenses.

To date, three animals have been immunized with the Case-A2 V1/V2 fusion protein, and one animal with a control immunogen consisting of the gp70-derived portion of the fusion protein, p621. The antigens were formulated with Ribi RAS adjuvant and administered by subcutaneous injection at an initial dose of 5 μg/Kg, followed by a boost after 1 month at a dose of 1 μg/Kg. Bleeds were taken at the day of and one week after the initial immunization, and at weekly intervals following the boost. Antibodies reactive with the immunogen were detected after the first boost. For the animal immunized with the gp70 fragment (p621) these antibodies were specific for the p621 protein, while the three animals immunized with the V1/V2 protein produced antibodies directed against both the gp70 portion and the V1/V2 domain.

These antibodies were further characterized by absorption of the gp70-specific fraction on a gp70 column followed by ELISA against several V1/V2 fusion proteins. As shown below (Fig. 7) for the animal immunized with the gp70 fragment (p621) these antibodies were all absorbed on an affinity column containing the immobilized p621 protein. For the three animals immunized with the V1/V2 fusion protein, the p621-absorbed antibodies retained reactivity for the Case-A2 V1/V2 protein, but not for the p621 gp70 fragment. All three of these sera recognized two heterologous proteins in addition to the Case-A2 sequence, a Brazilian clade protein (p580) and a Thai clade E protein (p599). The titers were higher for the autologous immunogen than for the nonautologous proteins, and lowest for the more distant clade E protein. However, this crossreactivity demonstrated that these macaques were producing a fraction of antibodies directed against highly conserved V1/V2 epitopes, in addition to antibodies restricted for the Case-A2 and related clade B proteins. Sera from the animal with the highest titer, #7026, also reacted weakly with the T15K peptide.

These immunizations are continuing, and we expect the antibody titers to increase with time. Additional characterizations of the epitope specificity of the resulting sera will be performed, and the neutralizing activities of the sera and specific antibodies isolated from these sera will be evaluated, as described above for the rat sera.

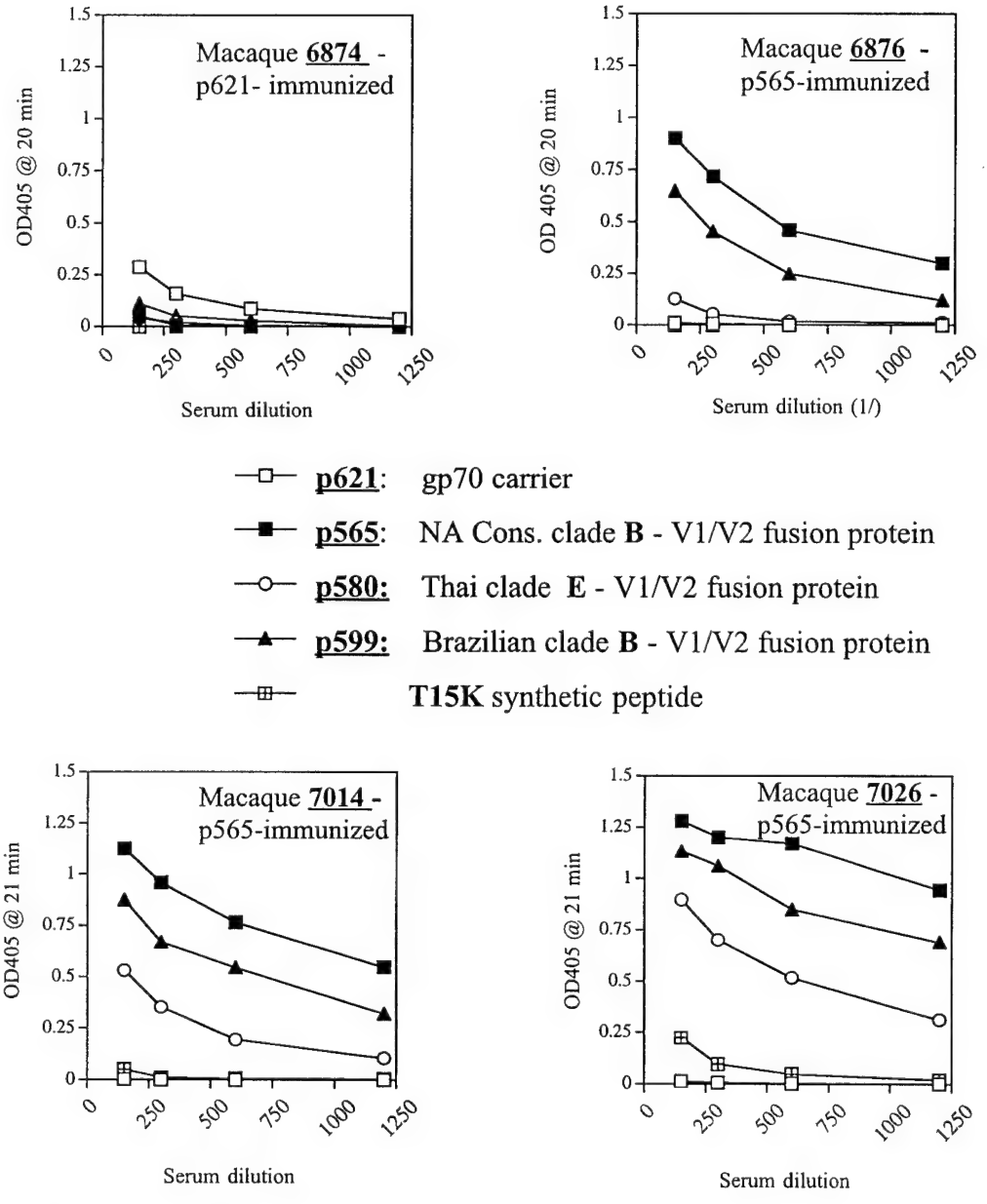


Fig. 7. Cross-clade immunoreactivity of sera of macaques immunized with either a gp70 fragment (p621) or gp70- Case-A2 V1/V2 fusion protein (p565). Sera were obtained 2 weeks post second immunization (week 6), depleted on p621 affinity column, and tested against various purified V1/V2 fusion glycoproteins and the T15K peptide by direct ELISA.

(OT4-276)

CONCLUSIONS

These studies have confirmed the potency of antibodies against native epitopes in the V1/V2 domain, and have demonstrated that our Case-A2 V1/V2 fusion protein is capable of inducing such antibodies. However, the yield of this class of antibodies has been low, while the majority of the antibodies produced were directed against epitopes that do not mediate neutralization and that may be carried on non-native forms of the immunogen. Our challenge is to learn how to modify the immunogen and/or immunization protocols so that the focus of the immune response is directed against the neutralizing targets.

During the coming year we shall extend these studies in the following directions.

1- We shall continue immunogenicity studies with our current V1/V2 prototype vaccine in rodents and macaque models. The rat studies will allow us to quickly evaluate different immunogen and adjuvant combinations, and to isolate additional monoclonal antibodies against specific V1/V2 epitopes. The macaque model has several advantages. First, it is important to determine whether primates exhibit a similar humoral response to this immunogen, before trials in humans can be considered. The macaque model will allow us to test this, and also allow the evaluation of the protective effects of these immune, using appropriate SHIV challenges. This animal model will allow the determination of the most effective adjuvants for inducing these antibody responses, and may also allow the determination of whether these vaccines induce CTL responses. Secondly, a difficulty in fully evaluating the activities of the immune rat sera is the limited amounts of sera that can be obtained from these animals. The macaques are much larger animals, and can therefore provide sufficient antisera for multiple analyses and fractionations, by ourselves as well as by independent laboratories. The macaques being used in these experiments are being provided by an NIAID-funded program. This has allowed us to rebudget the funds originally intended for primate studies towards supporting additional personnel to work on optimizing antigen design.

We will repeat the studies described above in larger scale and in additional animals, to fully characterize the potency and breadth of neutralizing activity of the anti-V1/V2 antibodies induced by the Case-A2 V1/V2 immunogen, and to see how common such responses are. If antibodies with similar activities can be induced in monkeys, this will facilitate the production of sufficient quantities of sera to carry out large scale assays against multiple isolates, including a broad range of clinical isolates and non-clade B viruses. Newer immunogens we anticipate to test will include V1/V2 constructs derived from non-clade B sequences and modified immunogens in which the gp70 sequences have been removed and replaced with appropriate T helper epitopes. We also expect to initiate studies of composite immunogens, including mixtures of different V1/V2 sequences and immunogens containing both V1/V2 and V3 sequences linked in several ways. Depending on the results of the epitope mapping studies designed to distinguish neutralizing from non-neutralizing epitopes, we may also be able to design and test second generation immunogens which have been optimized for the retention of neutralization epitopes

2- Additional future directions we intend to take this study include the improvement of the Case-A2 V1/V2 immunogen to focus the humoral responses towards the conformational epitopes that induce the most potent neutralizing responses. This will involve evaluation of alternate strategies to either remove or modify the gp70-derived fusion sequences, to avoid the generation of antibodies against the carrier sequences. More importantly, our studies have shown that the V1/V2 domain is an immunologically complex structure. The most potently neutralizing antibodies are directed against the conserved conformational epitopes, and these are the most relevant ones for protection. A second

class of antibodies are directed against the T15K V2 linear epitope. Whereas these also neutralize many viruses, they are less potent. One possibility is that mutating this sequence may increase the immunogenicity of the conformational epitopes. Previous studies with monoclonal antibodies to V1/V2 have shown that most of these have little, if any neutralizing activity. Such antibodies, if present in excess may be deletorious, since they may block the interaction of the neutralizing antibodies, thereby inhibiting their activities. Finally, we have found in some human sera the presence of anti-V1/V2 antibodies that actually enhance the infection by some strains of HIV-1. Such antibodies could be harmful if induced in response to immunization.

We are currently performing mutagenesis studies of our V1/V2 fusion protein to define the role of individual residues in determining folding, immunoreactivity and immunogenicity of this protein. Ideally, we would like to map the different classes of epitopes. This would allow us to hopefully modify the immunogen so that the deletorious epitopes are eliminated, while retaining and perhaps enhance the immunogenicity of the protective epitopes. The broad crossreactivity and potent cross-neutralizing activities we have been able to induce in rodents with our current vaccine is very encouraging, and suggests that this vaccine, and improved versions derived from the current immunogen, may induce protective responses in humans as well.

REFERENCES

1. **Ashkenazi, A., D.H. Smith, S.A. Marsters, L. Riddle, T.J. Gregory, D.D. Ho, and D.J. Capon.** 1991. Resistance of primary HIV-1 isolates to soluble CD4 is independent of CD4-gp120 binding affinity. Proc. Natl. Acad. Sci. USA, **88**: 7056-7060.

2. **D'Souza, M.P., G. Milman, J.A. Bradac, D. McPhee, C.V. Hanson, R.M. Hendry, and a.C. Investigators.** 1995. Neutralization of primary HIV-1 isolates by anti-envelope monoclonal antibodies. AIDS, **9**: 867-874.

3. **Golding, H., M.P. D'Souza, J. Bradac, B. Mathieson, and P. Fast.** 1994. Neutralization of HIV-1: Meeting report. AIDS Res. Hum. Retroviruses, **10**: 633-643.

4. **Gomatos, P.J., N.M. Stamatos, H.E. Gendelman, A. Fowler, D.L. Hoover, D.C. Kalter, D.S. Burke, E.C. Tramont, and M.S. Meltzer.** 1990. Relative inefficiency of soluble recombinant CD4 for inhibition of infection by monocyte-tropic HIV in monocytes and T cells. J. Immunol., **144**: 4183-4188.

5. **Guo, M.M.L. and J.E.K. Hildreth.** 1995. HIV acquires functional adhesion receptors from host cells. AIDS Res. Hum. Retroviruses, **11**: 1007-1013.

6. **Hanson, C.V.** 1994. Measuring vaccine-induced HIV neutralization: Report of a Workshop. AIDS Res. Hum. Retroviruses, **10**: 645-648.

7. **Hildreth, J.E.K. and R.J. Orentas.** 1989. Involvement of a leukocyte adhesion receptor (LFA-1) in HIV-induced syncytium formation. Science, **244**: 1075.

8. **Honnen, W.J., Z. Wu, S.C. Kayman, and A. Pinter**. *Potent neutralization of a macrophage-tropic HIV-1 isolate by antibodies against the V1/V2 domain of gp120.* Vaccines 1996: Molecular Approaches to the Control of Infectious Diseases, E.N. F. Brown, D. Burton, and J. Mekalanos, eds. Cold Spring Harbor Laboratory Press. 289-297.

9. **Kayman, S.C., Z. Wu, K. Revesz, H.-C. Chen, R. Kopelman, and A. Pinter.** 1994. Presentation of native epitopes in the V1/V2 and V3 domains of HIV-1 gp120 by fusion glycoproteins containing fragments of gp120. J. Virol., **68**: 400-410.

10. **Kostrikis, L.G., Y. Cao, H. Ngai, J.P. Moore, and D.D. Ho.** 1996. Quantitative analysis of serum neutralization of human immunodeficiency virus type 1 from subtypes A, B, C, D, E, F and I: lack of direct correlation between neutralization serotypes and genetic subtypes and evidence for prevalent serum-dependent infectivity enhancement. J. Virol., **70**: 445-458.

11. **Mascola, J., O. Weislow, S. Snyder, S. Belay, M. Yeager, F. McCutchan, J. McNeil, D. Burke, and M.C. Walker.** 1994. Neutralizing antibody activity in sera from human immunodeficiency virus type 1 vaccine recipients from the AIDS Vaccine Clinical Trials Network. AIDS Res. Hum. Retroviruses, **10:(Suppl 2)**: S55.

12. **Matthews, T.J.** 1994. Dilemma of neutralization resistance of HIV-1 field isolates and vaccine development. AIDS Res. Hum. Retroviruses, **10**: 631-632.

13. **McKeating, J.A., C. Shotton, J. Cordell, S. Graham, P. Balfe, N. Sullivan, M. Charles, M. Page, A. Blomstedt, S. Olofsson, S.C. Kayman, Z. Wu, A. Pinter, C. Dean, J. Sodroski, and R.A. Weiss.** 1993. Characterization of neutralizing monoclonal antibodies to linear and conformation-dependent epitopes within the first and second variable domains of human immunodeficiency virus type 1 gp120. J. Virol., **67**: 4932-4944.

14. **Moore, J., Y. Cao, L. Qing, Q.J. Sattentau, J. Pyati, R. Koduri, J. Robinson, C.F.B. III, D.R. Burton, and D.D. Ho.** 1995. Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120, and their neutralization is not predicted by studies with monomeric gp120. J. Virol., **69**: 101-109.

15. **Moore, J.P., Q.J. Sattentau, H. Yoshiyama, M. Thali, M. Charles, N. Sullivan, S.-W. Poon, M.S. Fung, F. Traincard, M. Pincus, G. Robey, J.E. Robinson, D.D. Ho, and J. Sodroski.** 1993. Probing the structure of the V2 domain of human immunodeficiency virus type 1 surface glycoprotein gp120 with a panel of eight monoclonal antibodies: human immune response to the V1 and V2 domains. J. Virol., **67**: 6136-6151.

16. **Pinter, A., W.J. Honnen, S.C. Kayman, O. Troshev, and Z. Wu.** 1997. The V1/V2 domain of gp120 is a sensitive neutralization target for primary, macrophage-tropic HIV-1 isolates. Submitted, .

17. **Sawyer, L.S.W., M.T. Wrin, L. Crawford-Miksza, B. Potts, Y. Wu, P.A. Weber, R.D. Alfonso, and C.V. Hanson.** 1994. Neutralization sensitivity of human immunodeficiency virus type 1 is determined in part by the cell in which the virus is propagated. J. Virol., **68**: 1342-1349.

18. **Wang, N., Z. Wu, W.J. Honnen, S.C. Kayman, D.D. Ho, and A. Pinter.** 1997. Potent neutralization of autologous primary HIV-1 isolate by anti-V1/V2 antibody fraction of a patient serum. In prep.

19. **Wang, N., T. Zhu, and D.D. Ho.** 1995. Sequence diversity of V1 and V2 domains of gp120 from human immunodeficiency virus type 1: lack of correlation with viral phenotype. J. Virol., **69**: 2708-2715.

20. **Warrier, S., A. Pinter, W.J. Honnen, M. Girard, E. Muchmore, and S.A. Tilley.** 1994. A novel glycan-dependent epitope in the V2 domain of HIV-1 gp120 is recognized by a highly potent neutralizing chimpanzee monoclonal antibody. J. Virology, **68**: 4636-4642.

21. **Wu, Z., S.C. Kayman, H.C. Chen, S. Warrier, S.A. Tilley, J. McKeating, C. Shotton and A. Pinter**. 1995. Characterization of neutralization epitopes in the V2 region of HIV-1 gp120: role of conserved glycosylation sites in the correct folding of the V1/V2 domain. J. Virol., **69**: 2271-2278.

Bibliography of publications and meeting abstracts published in 1996-7

1. W.J. Honnen, Z.Wu, S.C. Kayman and A. Pinter. 1996. Potent neutralization of a macrophage-tropic HIV-1 isolate by antibodies against the V1/V2 domain of gp120. Vaccines 1996: Molecular Approaches to the Control of Infectious Diseases. pp. 289-297.

2. S. Vijh-Warrier, A. Pinter, W.J. Honnen and S.A. Tilley. 1996. Synergistic neutralization of human immunodeficiency virus type 1 by a chimpanzee monoclonal antibody against the V2 domain of gp120 in combination with monoclonal antibodies against the V3 loop and the CD4-binding site. J. Virol. 70, 4466-4473.

3. S.H. Pincus, K. Wehrly, R. Cole, H. Fang, G.K. Lewis, J. McClure, A.J. Conley, B. Wahren, M.R. Posner, A.L. Notkins, S.A. Tilley, A. Pinter, L. Eiden, M. Teintze, D. Dorward and V.V. Tolstikov. 1996. Characterization of the in vitro effects of a panel of anti-HIV immunotoxins directed against multiple epitopes on gp160. AIDS Res. Hum. Retroviruses, 12, 1041-1051.

4. A. Pinter, W.J. Honnen, S.C. Kayman, O. Troshev and Z. Wu. 1996. The V1/V2 domain of gp120 is a sensitive neutralization target for primary, macrophage-tropic HIV-1 isolates. Submitted.

5. O. Alsmadi, E. Murphy, R. Herz, A. Pinter and S.A. Tilley. 1996. Two monoclonal antibodies isolated over a year apart from an asymptomatic HIV-1-infected individual recognize a novel, conserved antibody-dependent cellular cytotoxicity epitope in gp120. Submitted.

6. Ditzel, H. J., P. W. H. I. Parren, N. Sullivan, J. Sodroski, L. S. W. Sawyer, R. M. Hendry, N. Dunlop, P. L. Nara, A. Trkola, J. P. Moore, A. Pinter, C. F. B. III and D. R. Burton. 1996. A recombinant human Fab fragment directed against a conformation dependent epitope in the second variable region of HIV-1 gp120 neutralizes T-cell line adapted strains of HIV-1 but primary isolates less efficiently. Submitted.

Meeting Abstracts

1. W.J. Honnen, Z. Wu, S.C. Kayman and A. Pinter. The V1/V2 domain of HIV-1 gp120 contains potent neutralization targets for macrophage-tropic HIV-1 isolates. Molecular Aspects of the Control of Infectious Diseases, Cold Spring Harbor, N.Y., September 1995.

2. A. Pinter, W.J. Honnen, Z. Wu, O. Troshev and S.C. Kayman. Identification of highly conserved epitopes in the V1/V2 domain of HIV-1 gp120 that mediate potent neutralization of macrophage-tropic primary isolates. Conf. on Advances in AIDS Vaccine Development, Eighth Annual NCVDG Meeting, Wash. DC Feb. 12, 1996.

3. A. Pinter, W.J. Honnen, Z. Wu, O. Troshev and S.C. Kayman. Antibodies in human sera to highly conserved epitopes in the V1/V2 domain of gp120 that mediate potent neutralization of macrophage-tropic HIV-1 isolates. XIth Intl. Conference on AIDS, Vancouver, Canada, July 1996.